The "bacterial symbiosis" in the concretion deposits of certain operculate land mollusks of the families cyclostomatidae and annulariidae

Meyer, K F

DOI: https://doi.org/10.1093/infdis/36.1.1

THE "BACTERIAL SYMBIOSIS" IN THE CONCRETION DEPOSITS OF CERTAIN OPERCULATE LAND MOLLUSKS OF THE FAMILIES CYCLOSTOMATIDAE AND ANNULARIIDAE

K. F. MEYER

From the laboratories of the Zoologisch,-vergleichend-anatomischen Institut der Universität Zürich, the Hygiene Institut der Eidgen. Technischen Hochschule Zürich and the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco, California, U. S. A.

INTRODUCTION

Claparède described in 1858 a peculiar organ situated in the connective tissue of the dorsal region of the prosobranch mollusk, Cyclostoma elegans. This "glande" surrounding the intestines and boarding the nephridium consisted of snow-white, roundish granules. These had been noted as early as 1845 by Brard, who reported at that time that Cyclostoma elegans harbored a large quantity of small, "yellowish" calciferous granules, which were irregularly distributed throughout the connective tissue. Claparède made microchemical tests with the concretions, but failed to establish calcium as the main constituent. He was convinced that the organ had not been observed in other mollusks and designated it as "glande à concrétions." Garnault (1877) in the course of his careful studies on the anatomy and histology of Cyclostoma elegans devoted considerable attention to this "glande." According to this author the organ consists of a meshwork of closed tubules varying in length, but not exceeding 2 mm., the lumen of the tubules is filled with concretions, surrounded by clumps of rod-like bodies, which he recognized as bacilli. He attempted their cultivation by the methods, which had been introduced by Robert Koch about that time.

As recently as 1907, Barbieri discussing the concretions of Cyclostoma refused to accept Garnault's interpretation. Incidental to an histologic study of the embryo of this mollusk he made comments on the "glande à concrétions" and the rod-like elements, which it contained. He considered them to be small mineral concretions, probably phosphates. Unfortunately, no microchemical or bacterioscopic tests were reported to uphold his contention. The nature of these so-called bacteria was studied by Mercier (1911 and 1913) in connection with his work on the symbiotic micro-organisms of invertebrates. This investigator in a preliminary note, in 1911, accepted the view of Garnault and considered the rods of the "glande à concrétions" bacilli. He published, in 1913, his detailed studies and illustrated his observations by excellent plates. He showed in contrast to the findings of Garnault that the bacteria and concretions, which consisted of purine bases, namely uric acid, were not free in the tubules of a gland, but were enclosed in peculiar groups of cells located in the peri-intestinal connective tissue. These findings bring to mind numerous similar observations that have been made on bacteriods, mycorhizas, mycetomas of plants and animals, and it is from this point of view that additional studies appear desirable.

M. Portier's treatise "Les symbiotes" (1918) and Buchner's (56) monograph "Tier and Pflanze in intracellularer Symbiose" 1921, have recently aroused new

Received for publication, June 2, 1924.

interest in a relatively virgin field of investigation. Portier favors the conception that mitochondria are symbiotic micro-organisms. His contention is based on a very careful review of the literature, but his comparison between mitochondria and bacteria is lacking in directness. His interpretation has been upheld by his colleague Bierry and severely criticized on general grounds by Regaud, Guillermond and Laguesse. Auguste Lumière in his treatise "Le mythe des symbiotes" analyzes by means of bacteriological tests the claims of Portier. He concludes that the cells of normal animals do not contain micro-organisms, but that the tissues of vertebrates may enclose the spores of latent saprophytic bacteria. These will develop into active cultures when fragments of organs are transferred to nutritive mediums. The examples of symbiosis, which are observed in nature, are always evidence of a struggle between cell and parasite. There does not exist a true equilibrium between the two elements. Lumière concludes that mitochondria should not be confused either with bacteria or with the vitamines, which as chemical subtances are poorly defined, and not identical with the symbiotes.

Meves (1918) has advanced the view independently of Portier that mitochondria are widely adapted symbiotic bacteria. He emphasizes the fact that one should always realize that the cell is the result of a progressive evolution.

Wallin (1922) has recently published observations on the staining of bacteria with mitochondrial methods and on the reactions of bacteria to chemical treatment. These have led him to advance an hypothesis similar to that of Portier, i. e., that all mitrochondria are symbiotic bacteria. Whereas the evidence presented by Portier and Wallin is not convincing, it has a distinct bearing on the conception of the significance of the intracellular bacillary bodies in the concretion deposits of Cyclostoma elegans. If these views are correct, the identification of obscure intracellular elements cannot be established by accepted microscopical and bacteriological methods.

In this connection it appears appropriate to illustrate by a few examples selected from the literature the difficulties which have been encountered in deciding the exact nature and classification of the elements found in certain cells or groups of cells. A careful review of Buchner's book furnishes an endless list, but for the sake of brevity three interesting examples are given.

(1) The bacillary shaped organisms which are found in the root-nodules of leguminosae have received various interpretations. Beijerinck,[18] Frank, Laurent and others were able to demonstrate that the rod-like elements are foreign to the plant tissue, may be cultivated in artificial mediums and may infect young plants grown on sterile soil. Until these facts were established the conception that these organisms were simple modifications of the cytoplasm had a number of adherents (Brunchorst, Vuillemin). (2) Blochmann reported in 1887 the presence of rod-like elements in the fat body cells of certain Blattidae (Periplaneta orientalis). He insisted on the great resemblance of these bodies to that of bacterial micro-organisms, and in 1892 declared them to be symbiotic bacteria, a view also shared by Forbes (1892). Subsequently, Cuénot (1892), Prenant (1904), Henneguy and C. K. Schneider concluded that the questionable inclusions were products of metabolism and not bacteria. These tiresome discussions were apparently put to end when Mercier (1906) announced the cultivation of the so-called "Blochmann bodies." He grew these organisms on nutrient agar, gelatine, potatoe, milk and bouillon and named them Bacillus cuenoti. Recent studies by Javelly, Hertig and Glaser support the original interpretation of Blochmann, but neither of the workers has thus far succeeded in propagating the true symbiote* on artificial mediums. The exact nature of the symbiotic

* Symbiont is a misnomer; the Greek work for "companion" or "partner" is symbiote.

organisms found in the fat body and the polar mass in the eggs of certain scale insects is equally uncertain. (3) The cellular elements, which have been observed by Buchner, Sulc and Pierantoni in the tissues of coccids and in other homoptera have usually been interpreted as yeast-like organisms, which are commonly referred to as the genus Saccharomyces or allied genera located in the same group of plants. Berlese (1905) has placed the organisms which he cultivated from Ceroplastes in the genus Oöspora, thus recognizing it as a true fungus (Hyphomycetes). Brues and Glaser have shown that the symbiote of Pulvinaria innumerabilis, which they succeeded in cultivating on artificial mediums cannot be regarded as a saccharomyces, but as a representative of the species "Dematium" or a related genus, although its morphology and method of multiplication in the insect are similar to those of a saccharomycete.

These examples will emphasize sufficiently the difficulties encountered in the identification of certain cellular elements. Furthermore, it is evident that morphological studies, staining reactions and the behavior toward chemicals are uncertain criteria in distinguishing intracellular metabolic products of animal and plant tissues from symbiotic bacteria, yeasts and fungi. Unstained specimens furnished the best information regarding the bacterial nature of intracellular bodies (Mercier, Buchner and others). This method of examination is unfortunately not always possible, and the investigator is dependent on fixed and stained preparations. Crystalloids, mitochondria, albuminous granulations, etc., present pictures, which are frequently mistaken for bacteria or yeasts. The following example cited by Mercier (1913, p. 7) needs no comment. Cuénot (85) observed certain connective tissue cells in the earthworm, filled with small colorless crystals. These were misinterpreted by Lortel and Despergins as "tubercle bacilli" ingested by the worm living in soil contaminated with sputum! Comparative morphologic study may, however, furnish valuable information. It is possible, in some cases, to follow the development of the crystalloids in the cells. Prenant, Limon and Legendre have pointed out that the crystals observed in the animal kingdom show inconstancy in form and arrangement, while the character of the intracellular bacteria and yeasts is remarkably constant. Opportunity will be afforded to discuss this statement in the course of this paper.

Microchemical tests furnish information of only relative value as Prenant pointed out in the sentence: "la microchimie n'en est encorse qu' à la période d'essai." Crystals are frequently stained by aniline dyes; for example, those in the connective tissue of the earthworm take the Gram and the acid-fast stain as readily as tubercle bacilli (Cuénot, 1898). The crystalline formations in the liver of Sphaeronia and of Gyge observed by Bellonci and Emery are soluble in acid and alkalis. They are stained with iodine and therefore resemble the Rhizobium found in root-nodules of leguminous plants. Tests for solubility or the optical behavior of the bacillary intracellular elements in polarized or fluorscopic light are rarely conclusive, as Mercier has pointed out in a number of excellent examples.

It must therefore be concluded that the propagation of intracellular bodies on artificial mediums constitutes the only decisive argument whereby they can be designated either as living micro-organisms, parasites or symbiotes. When yeasts or algae are present, the identification of the cultures is relatively easy. However, enormous difficulties may arise when the symbiotes belong to the polymorphic group of bacilli or bacteria. Under these circumstances it is important to test the physiological activities of the isolated organisms and to prove their parasitic or symbiotic properties by experimental inoculations. Such a procedure has been employed with great success by Noel Bernard, Magrou and others in the study of the fungi involved in the tuberization of various plants like orchids

and Solanum tuberosum. Such a mode of experimentation is, however, impossible when the infection and transmission of the symbiotes is maintained by an hereditary process. According to Blochmann, Henneguy, Mercier, Lindner, Sulc, Pierantoni, Buchner and others, most of the symbiotes infecting the insects are transmitted from generation to generation through the egg in a very definite manner. Even bacteria, which exist in the leave-nodules of Ardisia crispa are transmitted through the seed according to Miehe. The hereditary transmission of the symbiotic micro-organisms explains their constant association with every individual of a particular species of host, but nullifies any attempt to test the cultivated micro-organisms by inoculating a host already contaminated through the egg.

The symbiotes from a small number of host species have been propagated on artificial mediums, but in most cases the cultivation experiments have met with unsurmountable difficulties. This may be due to the fact that the exact physiological conditions existing in the cells, the mycetomes or the bacteriocytes are still unknown. Analyzing the available literature, so ably summarized by Buchner, one realizes that the bacteriologist and the mycologist have found new fields for investigation. One gains the impression that a careful study of intracellular symbiosis in invertebrates may fertilize the field of pathology and immunology. The recent observations of Buchner on the constant occurrence of symbiotes in the mycetomes of blood-sucking insects like culex, anopheles, pediculi, etc., may have a distinct bearing on the conception of the transmission of pathogenic micro-organisms by insects. Equally novel and fascinating are the studies by Pierantoni, Harvey and others on the relation of bacteria to animal light. In the latter field, particularly, carefully controlled bacteriological tests may suggest numerous new problems.

The study of the concretion deposits of Cyclostoma elegans presents a number of intricate problems. Although the investigation is not complete, it appears advisable to report on the following phases thus far completed:

(1) A verification of the microscopic findings reported by Claparède, Garnault and Mercier on Cyclostoma elegans.

(2) An anatomical and histological study of the concretion deposits and nephridium of Cyclostoma lutetianum, sulcatum and mauretanicum * Leonia mamillare, Tudora putre, Adamsiella variabilis and Chondropoma subreticulatum, majusculus and dentatum.

(3) Bacteriological studies, an attempt to cultivate the intracellular bacteria of Cyclostoma elegans, lutetianum, sulcatum and Leonia mamillare.

(4) A biochemical and serological study of the predominant bacteria isolated from Cyclostoma elegans.

(5) Physiological studies on Cyclostoma elegans and the function of the concretion deposits.

* Since the foregoing has been written, a few specimens of Cyclostoma Olivieri Sowerby (Lebanon, Syria) and Tudorella ferruginea Lamarck (Balearic Isles) have been examined. They also possess concretion deposits and purinocytes infected with bacteria. The detailed studies will be reported in a separate paper.